Research Article

RNA: DNA Ratio and Growth Performance of Rohu *Labeo rohita* (Hamilton) Fed Varied Proportion of Protein Diet during Intensive Aquaculture

Shyam Narayan Labh

Aquaculture Research Lab, Department of Zoology, Amrit Campus, Tribhuvan University, Kathmandu, Nepal.

**ABSTRACT**

An experiment was conducted at Corona of Agriculture (COA)*, Gunjanagar-3, Chitwan, Nepal to complete a project granted by University Grant Commission (UGC), Second Higher Education Project (SHEP) in 2013. Four (average 380 m²) ponds (W, X, Y and Z) with four different diets as D₁ ((20%), D₂ (30%), D₃ (40%) and D₄ (50%)) protein contents were used to conduct the experiment properly to determine the varied proportion of dietary protein on the growth performance of major carp rohu *Labeo rohita* (H) in relation with RNA: DNA ratio. After 12th weeks of culture, average length, average weight and specific growth rate of fish were found significantly (\(P<0.05\)) higher in the carp with D₃ and D₄ diet fed fish. Similar results were observed in average total protein, albumin and globulin contents. RNA content increased rapidly with age. The average RNA content increased highest in D₃ and D₄ diet fed fish while, DNA content were highest in D₀ diet fed fish. RNA: DNA ratio was recorded highest in D₀ and D₄ diet fed fish. RNA: DNA ratio, an indicator of protein synthesis and have been used to accurately estimate the growth rate and feeding condition of fish hence, as the dose of protein increased RNA and DNA contents also increased with age of carp cultured during experiment. Thus, it was clear from this study that the incorporation of protein in diet enhances the growth of fish regardless of species weight groups and the doses, as the average weight of fish was significantly lower in control diet fed fish as compared to the treated one.

**INTRODUCTION**

Aquaculture is a feed based industry with over 60% of the operational cost coming from feed sources alone[1] and fish farming is the cultivation of fish in captivity under controlled conditions. At present it is an important and rapidly expanding enterprise all over the world. In various parts of the world, fish farming is an extremely vital part of economy. In the developing countries, fishes are being raised as food and source of income[2], while in the more developed countries; fish produced is used primarily for restocking streams, lakes and rivers for sport fishing purposes[6]. The main purpose of fish farming is the production of white meat at large scale for human consumption[3]. Production can be realized through an increase in fish number and/or an increase in individual weight[8]. The cost of feed is largely influenced by the level and sources of protein which is the most expensive component of a fish diet. It is the major dietary component which influences growth of fish and insufficient as well as excess level of protein in feed is not desirable. Protein is the most important nutrient for fish growth and plays a central role in the structure and functioning of all living organism. According to Shang, (1996) fish is an important component of total human food consumption and a principal source of animal protein for more than half of the world’s population. Carps like all other animals must consume protein to maintain a continuous supply of amino acids. The consumed protein is digested or hydrolyzed to release free amino acids that are absorbed from the intestinal tract of the animal and distributed by blood to various organs[8].

RNA is directly involved in protein synthesis and therefore increases in RNA content are observed during periods of rapid growth, whereas DNA content is usually

*Corresponding author Email address: snlabh@gmail.com

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stability of RNA : DNA ratio an indicator of protein synthesis capacity per cell. RNA : DNA ratio is thus a frequently measured indicator of growth rate. RNA : DNA ratios were used successfully to predict growth and nutritional state in a multitude of studies on a variety of organisms such as bacteria, phytoplankton, insects, zooplankton, marine invertebrates, fish, reptiles, and humans. Thus, in the present study, rohu Labeo rohita (H) fed varied proportion of protein diets and then growth performance, protein profiles and RNA:DNA ratios were measured during intensive aquaculture.

MATERIALS AND METHODS

1. Formulation of Experimental Diets

Altogether four (40% crude protein) experimental diets containing protein percentages of 20%, 30%, 40%, and 50% had been used as Control diet (D1), Test diet (D2), Test diet (D3) and test diet (D4) respectively (Table-1). First of all fish meal, wheat flour, cod liver oil and multivitamins were collected from the local market to prepare experimental diets. To make fine powder fish meal was dried well and ground in a grinder and then sieved (mesh size: 500μ). The powdered fishmeal was mixed thoroughly with wheat flour and recommended multivitamin. Then lukewarm water was added in required amount for the formation of dough. Cod liver oil was added to this and mixed well so that all the ingredients were spread homogeneously. The prepared dough was passed through a feed maker using 1 mm die, the thread formed was air dried. The dried threads were further chopped into small pieces of required sizes of pellets through a blender and then passed through a sieve to obtain homogeneous particle size. Diet was stored at −20°C until used.

| Table 1 Ingredients composition (% in diet) for basal diet |
|-----------------|-------|-------|-------|-------|
| Ingredient (%)  | D1    | D2    | D3    | D4    |
| Fish Meal (g)   | 20    | 30    | 40    | 50    |
| Wheat Flour (g) | 17.7  | 17.7  | 17.7  | 17.7  |
| Ground Corn (g) | 49.3  | 39.3  | 29.3  | 19.3  |
| Rice Bran (g)   | 5     | 5     | 5     | 5     |
| Soya bean Cake (g) | 2  | 2     | 2     | 2     |
| Mustard Cake (g) | 2     | 2     | 2     | 2     |
| Vitamin (Premix) (g) | 1  | 1     | 1     | 1     |
| Cod Liver Oil (ml) | 3  | 3     | 3     | 3     |
| Total           | 100   | 100   | 100   | 100   |

2. Experimental Design and Feeding Trial

The experiment was conducted at the “Corona of Agriculture (COA)”, Gunjanagar-3, Chitwan with major carp, rohu Labeo rohita (H). Fish were cultured in outdoor natural conditions under four feeding regimes in order to find out the effects of varied proportion of protein content in food on the growth performance, protein profile and RNA:DNA ratio of the fish. Four different ponds (W, X, Y and Z) were used to conduct the experiment properly. The area of each pond was about 380m² so in each pond 3 small mess net cages (8x8x4) feet were used to make them replicates. The stocking density was 100 fish/pond i.e. 1 fish/m². The initial length of fish was 11.27 ± 0.08 cm and average initial body weight of individual fish was 16.25 ± 0.17 g. Thus Pond W was used for diet (D1) containing 20% protein (Dough form) used as control diet (W1, W2, and W3), Pond X used for diet (D2) containing 30% protein (X1, X2, and X3), Pond Y used for diet (D3) containing 40% protein (Y1, Y2, and Y3), and Pond Z used for diet (D4) containing 50% protein (Z1, Z2, and Z3) during the entire feeding trials.

Fish were fed twice daily at 9.00 AM and at 4.00 PM at the rate of 3% of their body weight. Water temperature, dissolved oxygen, free carbon dioxide, pH, and total alkalinity were measured by standard methods. The average water temperature was 26.0-27.5°C, dissolved oxygen 6.7-7.6 mg/l, free carbon dioxide 5-10 mg/l, pH 7.1-7.8, and total alkalinity 65-80 mg/l throughout the study period. Faecal matter and unconsumed feed, if any, were siphoned before feeding. The duration of the experiment was 3 months i.e. 12 weeks and samples had been taken fortnightly from the date of the experiment. Data for length and weight were recorded. On every week sample of fish were collected for the biochemical estimations and for this purpose selected fish were packed in vials in ice box from each pond and were kept to the laboratory of Department of Zoology, Amrit Campus. Fish were cultured in outdoor natural conditions under four feeding regimes in order to find out the effects of varied proportion of protein content in food on the growth performance, protein profile and RNA:DNA ratio of the fish. Four different ponds (W, X, Y and Z) were used to conduct the experiment properly. The area of each pond was about 380m² so in each pond 3 small mess net cages (8x8x4) feet were used to make them replicates. The stocking density was 100 fish/pond i.e. 1 fish/m². The initial length of fish was 11.27 ± 0.08 cm and average initial body weight of individual fish was 16.25 ± 0.17 g. Thus Pond W was used for diet (D1) containing 20% protein (Dough form) used as control diet (W1, W2, and W3), Pond X used for diet (D2) containing 30% protein (X1, X2, and X3), Pond Y used for diet (D3) containing 40% protein (Y1, Y2, and Y3), and Pond Z used for diet (D4) containing 50% protein (Z1, Z2, and Z3) during the entire feeding trials.

3. Examination Procedure

After the completion of experiment, fishes were collected from each pond. Difference in number of fish between the time at stocking and at harvest has been determined for estimation of fish survival. The length (cm) and weight (g) were measured and recorded using analytical balance. This has been expressed in percent of the initial number of fish. Length gain were measured by using formula; Length Gain (∂L) = Lt – Lo; Where Lt = Length during survey (after time t); Lo = Length during previous survey. Similarly, change in absolute body weight was determined by recording weight and using formula; Weight Gain (∂W) = Wt – Wo; Where Wt = Weight during survey (after time t); Wo = Weight during previous survey. The specific growth rate (SGR) was calculated using the formula: Specific Growth Rate (SGR) = 100 (ln Wt - ln Wo)/t Where, Wi and Wt were the initial and final body weights and t the time in days. And Feed conversion ratio (FCR) was calculated by using following formulae:

\[ \text{Feed Conversion Ratio (FCR)} = \frac{Fk}{(Wt- Wo)} \]
Amount of feed consumed by each fish during time period t, which was calculated by calculating the amount of feed given during certain time period divided by number of fish.

For biochemical studies, fish were collected from the experimental ponds and anesthetized with tricaine methanesulfonate (MS-222). 2 ml of blood sample was collected from the caudal vein of anesthetized fish with heparinized (21-gauge) disposable hypodermic needle. Collected blood was gently transferred into disposable plastic bottle containing dried heparin (20 IU/ml). The samples were then mixed gently and used for biochemical assays. Total protein of fish blood serum was determined by biuret method \(^{(23)}\) using kit. Albumin of fish blood serum was determined by BCG method \(^{(24)}\) using kit. Biochemical analyses for RNA content and DNA content estimation were done. Isolation of nucleic acids from the fish larvae were done by the method developed by \(^{(25)}\) Schneider (1945) and followed by \(^{(26)}\) Mustafa (1979). Estimation of RNA and DNA in fish larvae were done by method developed by \(^{(27)}\) Buckley and Bulow (1987). Estimation of DNA was done spectrophotometrically by Diphenylamine method. Estimation of RNA was done spectrophotometrically using Orcinol method.

At the end statistical analysis of data was done using one way ANOVA \(^{(28)}\) followed by Duncan’s Multiple Range Test \(^{(29)}\) at \(P<0.05\) level of significance. The data were processed as per SPSS software (version 15) to compare the target parameters.

**RESULTS AND DISCUSSION**

There was no significant (\(P>0.05\)) difference in the final average length of *Labeo rohita* (11.27±0.08 cm) among the treatments at the beginning of the study but after 12 weeks of culture, a direct relationship was found between the dose of protein and the final average body length of carp. The final average length was significantly (\(P<0.05\)) higher in the carp fed with diet D\(_1\) (23.77±0.029 cm) followed by fish fed with diet) D\(_2\) (23.51±0.016 cm), D\(_3\) (20.72±0.016 cm) and minimum in the group fed with dietD\(_4\) (19.08±0.014 cm), i.e. control diet (Figures 1 & 2). 131.5% increment were observed in final average length in D\(_1\) and D\(_2\) diet fed fish as compared to D\(_2\) (101.6%) and D\(_1\) (85.8%). Similar results were observed in final average weight of fish fed with control diet grew from 16.25 ± 0.17 g to 72.96 ± 1.33 g during the study period of twelve weeks, while the fish fed with D\(_1\) (40% protein) diet grew from 16.25±0.17 g to 219.74±0.27 g and those fed with D\(_3\) (50% protein) diet grew from 16.25±0.17 g to 219.81±0.44 g during the 12 weeks of study period (Figures 3 & 4). The specific growth rate was significantly less in the carp fed with diet D\(_1\) (1.78±0.09) and D\(_3\) (1.78±0.09) followed by fish fed with diet D\(_2\) (1.19±0.11) and minimum was in the control diet D\(_4\) (1.10±0.09) fed fish (Figures 5 & 6). Average specific growth rate of fish was found highest in fish fed with D\(_1\) (1.63) and D\(_3\) (1.45) as compared to fish fed D\(_1\) (0.98) and fish fed with D\(_1\) (0.84). Similarly, feed conversion ratio (FCR) of fish fed with different diets during the study period was calculated periodically and the lowest FCR was found in the fish fed with D\(_1\) diets. The average FCR of fish fed with diets D\(_0\), D\(_1\), D\(_3\) and D\(_4\) were 2.51, 2.10, 1.45 and 1.46 respectively (Figures 7 & 8).

Dietary protein is always considered to be of primary importance in fish feeding \(^{(30)}\), thus sufficient supply of dietary protein is needed for rapid growth \(^{(31)}\). In the present study, results revealed that the optimum dietary protein level was 30 to 40% for *Labeo rohita*. The high (D\(_1\)) protein level (50% protein) did not significantly enhance the fish growth. This result may be due to the fact that each fish size has a certain protein limit after which excess protein level could not be utilized efficiently. These results are in agreement with Tacon (1987) who reported that dietary protein level varies from 42% for fry to 35% for growing adult of omnivorous fish. The dietary protein requirement for fish fry was high and ranges from 35% to 56% \(^{(31)}\). Furthermore, Wilson (1989) and El-Sayed and Teshima (1991) found that dietary protein requirements decreased with increasing fish size and age. Based on studies \(^{(38)}\) made a general conclusion that fry of tilapia <1 g requires diet with 35-50% protein, 1-5 g fish requires diet with 30-40% protein and 5-25 g fish requires diet with 25-35% protein. These results may be due to the fact that each fish size has a certain protein limit after which excess protein level could not be utilized efficiently.

El-Sayed obtained conflicting results from their studies on the effect of dietary protein level on the growth of *Labeo rohita*. The dietary protein requirements of several species of fish have been estimated to range between 20% and 56% \(^{(37)}\). De Silva and Perera (1985), Siddiqui et al. (1988) and Abdelghany (2000) reported that the optimum dietary protein level for growth of fish fry was 30% crude protein. Hamza and Kenawy (1997) found out that 40% protein was more potent than other levels for Nile tilapia growth. Al-Hafedh (1999) and Al-Hafedh et al. (1999) found out that the better growth of fish was obtained at high dietary protein levels (40-45%) rather than 25-35%. Feed input is the single largest operational cost in majority of aquaculture practices \(^{(34)}\). De Silva and Gunasekera (1991) conclusively proved the existence of daily variations in dry matter and protein digestibility and opined that feeding fish everyday with the same level of protein is not economical.

Feed conversion ratio (FCR) decreased with increasing dietary protein level and the better FCR was found in group fed (D\(_3\) and D\(_4\)) when compared to control (D\(_1\)) fed group. These FCR trends are in agreement with that obtained by Seema, et al., (2001), Al-Hafedh (1999) and...
A significant increment showed in the average total protein contents in the beginning of the experiments i.e. from first week up to sixth week but after seventh week increase in protein contents was almost steady in all the ponds. The average total protein contents in the D1 diet fed fish was 124.50 ± 0.43 μg/mg in the beginning of the experiment, which increased into 155.11 ± 0.29 μg/mg in the 12th week of experiment. The highest increase in protein contents was found 176.98 ± 0.42 μg/mg (33.93%) in D1, diet fed fish followed by D3 with 33.46%, D2 with 26.80% and the least was in D4 with 24.59% and on an average it showed 24.59% increment in the average total protein contents at the end of experiment (Figures 9 & 10). The average albumin content of fish fed with control diet D1 increased from 85.793 ± 0.264 μg/mg to 103.777 ± 0.446 μg/mg compared to that of fish fed with D3 (30% protein) diet increased from 86.327 ± 0.069 μg/mg to 108.443 ± 0.139 μg/mg. D4 (40% protein) diet increased from 89.733 ± 0.266 μg/mg to 114.487 ± 0.084 μg/mg while that of fish fed with D4 (50% protein) diet increased from 91.473 ± 0.478 μg/mg to 117.397 ± 0.268 μg/mg. It was observed that highest 28.39% increase in average albumin content in D1, diet fed fish followed by 27.59%, 25.62% and 20.96% in D3, D4 and D1, diet fed fish respectively (Figures 11 & 12). Garg, et al. (2002) reported better results for Labeo rohita as compared to Cirrhinus mrigala when fed with similar diets to that used in their study. However, Khan, et al. (2003) has reported contrasting results and considered the high cost based diet (fish meal) as more effective for the better growth of Labeo rohita.

The average globulin content also varied significantly among the fish larvæ fed with different diets during the study period. The average globulin content of fish fed with control diet D1 was 38.707 ± 0.273 μg/mg in 1st week which increased up to 55.750 ± 0.650 μg/mg in 8th week and after 8th week and again decreased to 51.337 ± 0.623 μg/mg in the 12th week. Similarly, the average globulin content of fish fed with D3 (30% protein) increased from 38.833 ± 0.299 μg/mg to 58.653 ± 0.837 μg/mg in the 7th week and then decreased 50.253 ± 0.559 μg/mg in 12th week. Similar results were found in the average globulin content of fish fed with D4 (40% protein) and D1 (50% protein) diet fed fish. In D1 (40% protein) diet fed fish the average globulin contents was 40.470 ± 0.379 μg/mg increased up to 69.747 ± 0.105 μg/mg in 6th week and then decreased into 62.494 ± 0.500 μg/mg in 12th week while in D1 (50% protein) diet fed fish it was 40.851 ± 0.680 μg/mg in the beginning which increased up to 70.873 ± 0.614 μg/mg in 7th week and finally decreased into 59.202 ± 0.230 μg/mg in 12th week (Figures 13 & 14). A highest 54.42% average globulin contents was recorded in D1 (40% protein) diet fed fish as compared to D1 (44.92%) and D1 (32.63%). The lowest globulin concentration was found 29.41% in D1 (30% protein) diet fed fish. There were no significant differences found in the beginning and at the end of experiment for average albumin and globulin ratio (Figures 15 & 16).

The total protein represents the sum of albumins and globulins. Albumin is synthesized by liver using dietary proteins and its presence in the plasma creates an osmotic force that maintains fluid volume within the vascular space. The globulins are the proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins. Since, gamma fraction usually makes up the largest portion of the globulins, antibody deficiency should always come to mind when the globulin level is low. Serum albumin and globulin values in the fishes treated with different immune-stimulants were significantly higher than control. In the present study it was observed that average total protein content found highest 33.93% in D1 diet fed fish followed by D4, diet fed fishes with 33.46%, D2 diet fed fishes with 26.80% and the least was in D1, diet fed fishes only with 24.59% increase in protein contents. Similar result was observed in average albumin content whereas highest 28.39% increase in D1 diet fed fish followed by 27.59%, 25.62% and 20.96% in D3, D4, and D1 diet fed fish respectively. The present study was in conformity with the findings of Choudhury et al. (2005), Sahu et al. (2007), Wijendra et al. (2007), Kumar et al. (2012) and Kumar et al. (2013). The findings of Ali et al. (2005) demonstrated that the mixed feeding schedule of a Low Protein (LP) alternated with a High Protein (HP) resulted in the best growth, feed utilization and production compared with feeding sushi catfish and silver carp with a High Protein (HP) continuously. Similar types of growth response have also been reported in common carp, in which low and high protein based diets were fed alternately resulted in higher growth and this was considered as a possible way of reducing feed cost. Patel, 2003 also reported in the finding of alternate feeding schedule of two diets containing high (33%) and low (22%) protein. There was a significant improvement in growth performance and protein utilization parameters, such as apparent protein conversion efficiency and protein efficiency ratio against continuously high protein feeding. The considerable variations in the results recorded previously for optimum dietary protein requirements for maximum growth might be due to the variations in fish size and age, stocking density, protein quality, hygiene and
environmental conditions or other unknown factors, which mask the standardization of the parameters. Salim and Sheri (1999) observed significant influence of high protein diets (50%) on growth performance of *Labeo rohita* fingerlings followed by medium protein diets (45%) and low protein diet (40%) respectively[82].

RNA content increased rapidly with age. The average RNA content of fish fed with control diet was increased up to 94.04% from beginning 4.737±0.052 μg/mg in 1st week to 9.192 ± 0.095 μg/mg at the end of 12th week of experiment. RNA contents of D2, D3, and D5 diets fed fish were 4.739 ± 0.027 μg/mg to 9.270 ± 0.097 μg/mg, 5.338 ± 0.041 μg/mg to 9.349 ± 0.099 μg/mg and 5.540 ± 0.086 μg/mg to 9.428 ± 0.101 μg/mg respectively. Statistically, there is a significant difference in RNA concentration among fish fed with different diets. The RNA content increased highest 95.61% in D2 (30% protein) diet fed fish, 75.14% in D3 (40% protein) diet fed fish and 70.18% in D5 (50% protein) diet fed fish (Figures 17 & 18).

Similarly, average DNA content was 2.932 ± 0.007 μg/mg in D1 (control) group fed fish in the beginning of the experiment which increased up to 3.242 ± 0.005 μg/mg after 12th week. In D2 (30% protein), D3 (40% protein) and in D5 (50% protein) diet fed fish (Figures 19 & 20) DNA contents were 2.924 ± 0.007 μg/mg, 2.927 ± 0.007 μg/mg and 2.923 ± 0.009 μg/mg which increased up to 3.247 ± 0.005 μg/mg, 3.253 ± 0.005 μg/mg and 3.258 ± 0.005 μg/mg respectively. A highest 11.46% DNA content were increased in D5 (50% protein) diet fed fish during the study period. The average RNA:DNA ratio of fish fed with control diet D1 was 1.62 ± 0.014 in first week which increased up to 2.84 ± 0.026 in 12th week of feeding trial. Similarly, average RNA:DNA ratio of fish fed with D2 (30% protein), D3 (40% protein) and in D5 (50% protein) diet fed fish increased from 1.62 ± 0.011 to 2.85 ± 0.026, 1.82 ± 0.018 to 2.87 ± 0.027 and 1.90 ± 0.035 to 2.89 ± 0.028 at the end of experiment, respectively. A highest 75.93% RNA:DNA ratio was recorded in D5 (30% protein) diet fed fish while it was 75.31%, 57.69% and 52.11% in D1 (control) group, D3 (40% protein) and in D5 (50% protein) diet fed fish (Figures 21 & 22).

Nucleic acids play a major role in growth and development. The RNA concentration is a sensitive parameter to determine the growth rate of an organism because it is the organizer of protein synthesis. DNA concentration represents an index of cell numbers since cellular DNA content is insensitive to changes in environmental condition. The ratio of RNA to DNA is, therefore, a more accurate index of metabolic activity than RNA concentration alone because the number does not affect this ratio or size of the cells in tissue samples[83]. RNA and DNA are compounds found in all living organisms. DNA is the genetic template; its cellular concentrations may be related to cell size and be relatively insensitive to changes in environmental conditions. In contrast, RNA is involved in protein synthesis, which is required for growth. Its cellular concentration is highly dependent on the growth rate, which is determined in part by environmental conditions. As a consequence, DNA concentrations may be a good measure of living biomass and RNA:DNA ratios may be a good indicator of growth rates of fish. In this study the RNA content increased 95.61% in D1, (30% protein) diet fed fish, 75.14% in D2 (40% protein) diet fed fish and 70.18% in D3 (50% protein) diet fed fish while the RNA content was 94.04% in control (D1) diet fed fish. Similar trend were observed in DNA content, a highest 11.46% DNA contents were increased in D5 (50% protein) diet fed fish during the study period. RNA content increased rapidly with age. The amount of DNA, the carrier of genetic information, remains stable under changing environmental situations and has been used as an indicator of biomass[84]. The concentrations of RNA in a tissue provide an estimate of ribosome numbers. The changes in nutritional status lead to alterations in ribosome numbers. Measurements of RNA:DNA ratios can provide useful information about the nutritional status of animals[65][66]. There is usually a significant correlation between nutritional status, RNA:DNA ratios and rates of growth[67][68]. RNA:DNA ratios in rohu were positively correlated to the trends in growth as has been indicated by several workers[85][86][87][88]. Wilder and Stanley (1983) confirmed the relationship between growth and RNA: DNA ratios in brook trout, *Salvelinus fontinalis* and *Atlantic salmon, Salmo salar*.[23]. In the present study, the elevated RNA: DNA ratios were associated with higher levels of RNA and lower levels of DNA. Increased RNA: DNA ratios noticed in rohu corresponding to growth increment are indicative of higher protein synthesis which could be attributed to fermented diets containing more total free amino acids. Moreover, quantification of RNA: DNA ratios is a well-established approach that has been used extensively to examine approximate short-term growth of field-collected larval[74][75][76][77][78][79].

Unlike our study, many previous studies evaluated relationships between growth and RNA: DNA ratios after prolonged periods, e.g., 30-56 d[80][81]. Stierhoff et al. (2009) used RNA:DNA ratios to investigate the growth response of two juvenile estuarine species (weakfish *Cynoscion regalis* and summer flounder *Paralichthys dentatus*) to hypoxia in a coastal Delaware bay and found a strong relationship between RNA:DNA ratios and growth after a 7-d period. Stierhoff et al. (2009) also suggested a response of RNA: DNA ratios occurred after only 1 d without food[82]. The strong effect of temperature on young yellow perch RNA:DNA ratios makes it less straightforward to use this measure to index growth of fish that experience variable temperatures. Similar to conclusions of previous studies[83][84][85] and Stierhoff et al. 2009 suggest that it is important to consider temperature when interpreting RNA:DNA ratios of field collected fish.
Figure 1 Average length of *Labeo rohita* fed varied proportions of diet in the given week

Figure 2 Average length of *Labeo rohita* fed varied proportions of diet in the given week

Figure 3 Average weight of *Labeo rohita* fed varied proportions of diet in the given week

Figure 4 Average weight of *Labeo rohita* fed varied proportions of diet in the given week

Figure 5 Specific Growth Rate of *Labeo rohita* fed varied proportions of diet in the given week

Figure 6 Specific Growth Rate of *Labeo rohita* fed varied proportions of diet in the given week

Figure 7 Feed Conversion Ratio of *Labeo rohita* fed varied proportions of diet in the given week

Figure 8 Feed Conversion Ratio of *Labeo rohita* fed varied proportions of diet in the given week
Figure 9 Average total protein of *Labeo rohita* fed varied proportions of diet in the given week

Figure 10 Average total protein of *Labeo rohita* fed varied proportions of diet in the given week

Figure 11 Average albumin of *Labeo rohita* fed varied proportions of diet in the given week

Figure 12 Average albumin of *Labeo rohita* fed varied proportions of diet in the given week

Figure 13 Average globulin of *Labeo rohita* fed varied proportions of diet in the given week

Figure 14 Average globulin of *Labeo rohita* fed varied proportions of diet in the given week

Figure 15 A/G ratio of *Labeo rohita* fed varied proportions of diet in the given week

Figure 16 A/G ratio of *Labeo rohita* fed varied proportions of diet in the given week
Figure 17 Average RNA concentration of *Labeo rohita* fed varied proportions of diet in the given week.

Figure 18 Average RNA concentration of *Labeo rohita* fed varied proportions of diet in the given week.

Figure 19 Average RNA concentration of *Labeo rohita* fed varied proportions of diet in the given week.

Figure 20 Average RNA concentration of *Labeo rohita* fed varied proportions of diet in the given week.

Figure 21 Ratio of RNA and DNA of *Labeo rohita* fed varied proportions of diet in the given week.

Figure 22 Ratio of RNA and DNA of *Labeo rohita* fed varied proportions of diet in the given week.
CONCLUSION

RNA: DNA ratio, an indicator of protein synthesis and have been used to accurately estimate the growth rate and feeding condition of fish hence, RNA and DNA contents increased rapidly with age of carp and the average RNA: DNA ratio increased up to 75.93% during the experiment. However, in case of RNA content increasing trend was observed with the increase in dietary protein level. Similarly as DNA content had no effect on dietary protein level. RNA: DNA ratio also followed the same increasing trend as RNA content along with the increase in dietary protein level during the whole experimental period. Thus, the outcomes from the 12 weeks of feeding trials indicated a varied growth rate under different (D1 to D5) treatments and D5 diet fed fish showed significantly (P<0.05) higher growth among the treatments and will be suitable for proper fish growth and can be recommendable to the fish farmers in tropical environment. Increase in the RNA: DNA ratio in recovering fishes can be considered as an indicator of protein synthesis and growth.

Based on the results obtained in this study it can be concluded that diet containing up to 40% dietary protein resulted in simultaneous increase in average length, average weight and specific growth rate and after that there was no change in all those growth parameters with increase in dietary protein level during the whole experimental period. Similarly, in case of feed conversion ratio there was decrease in FCR with the increase in dietary protein level up to 40% (D5). Increase in dietary protein level up to 40% (D5) the total protein content, albumin content and globulin content increased significantly. This was also clear from this study that the incorporation of protein in diet enhances the growth of fish regardless of species weight groups and the doses, as the average weight of fish was significantly lower in control diet fed fish as compared to the treated one. Number of larger weight group of fish were more in protein incorporated diet fed fish, compared to the control diet fed fish.

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